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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Deborah Knutzon et al.		
Assignee:	Calgene LLC /Abbott Laboratories		
Filing Date:	August 5, 1999	Examiner:	Nashed, Nashaat T.
Serial No.:	09/367,013	Art Unit:	1652
Title:	METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE AND AMENDMENT

Sir:

In response to the Office Action of December 31, 2002 ("Office Action"), reconsideration is requested in light of the following. A three month extension of time is requested.

IN THE CLAIMS

The following is a clean version of the amended claims. Please amend the following claims as indicated, and add new claim 297. An attachment is provided showing the revisions, captioned **"Version with markings to show changes made."**

189. (Amended) The method of claim 193, wherein the recombinant nucleic acid comprises the sequence depicted in SEQ ID NO: 1.

193. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a microbial cell comprising a recombinant nucleic acid with at least 80% homology to the sequence depicted in SEQ ID NO: 1 to produce the microbial cell culture, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

201. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a microbial cell comprising a recombinant nucleic acid operably linked to transcription and translation control signals functional in said cell to produce the microbial cell culture, wherein said nucleic acid is a deletion mutant of the nucleic acid depicted in SEQ ID NO: 1, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

205. (Amended) The method of claim 208, wherein the polypeptide comprises the amino acid sequence depicted in SEQ ID NO:2.

208. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a recombinant microbial cell comprising a polypeptide with at least 80% homology to the sequence depicted in SEQ ID NO: 2 to produce the microbial cell culture,

wherein said polypeptide forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

214. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a microbial cell comprising a recombinant nucleic acid to produce the microbial cell culture, wherein said nucleic acid hybridizes preferentially to the complement of the sequence depicted in SEQ ID NO: 1 under hybridization conditions suitable for sequencing said complement, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

215. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from a microbial cell culture produced by culturing a microbial cell comprising a recombinant nucleic acid comprising the sequence depicted in SEQ ID NO: 1, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid is expressed in sufficient amount in said culture to alter the fatty acid profile.

225. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from a microbial cell culture produced by culturing a microbial cell comprising a recombinant nucleic acid with at least 80% homology to the sequence depicted in SEQ ID NO: 1, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

235. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from a microbial cell culture produced by culturing a microbial cell comprising a recombinant nucleic acid operably linked to

transcription and translation control signals functional in said cell to produce the microbial cell culture, wherein said nucleic acid is a deletion mutant of the nucleic acid depicted in SEQ ID NO: 1, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

255. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from a microbial cell culture produced by culturing a recombinant microbial cell comprising a polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

265. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from a microbial cell culture produced by culturing a recombinant microbial cell comprising a polypeptide with at least 80% homology to the sequence depicted in SEQ ID NO: 2 to produce the microbial cell culture, wherein said polypeptide forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

275. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from a microbial cell culture produced by culturing a microbial cell comprising a recombinant nucleic acid to produce the microbial cell culture, wherein said nucleic acid hybridizes preferentially to the complement of the sequence depicted in SEQ ID NO: 1 under hybridization conditions suitable for sequencing said complement, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

Please cancel claims 291-296.

297. (New) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from a microbial cell culture produced by

culturing a microbial cell comprising a recombinant nucleic acid to produce the microbial cell culture, wherein said nucleic acid hybridizes preferentially to the complement of the sequence depicted in SEQ ID NO: 1 at 50 degrees Celsius in 60 mM Tris-Cl (pH 8.5) and 15 mM $(NH_4)_2SO_4$ and 2 mM $MgCl_2$, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

REMARKS

The pending claims have been amended to correct certain clerical errors, and to render certain dependent claims independent. Support for new claim 297 is set forth below. No new matter is added.

The Unity of Invention Objection

The Office Action stated that claims 185-214, 291 and 294 were not in Group I elected by Applicants. Cancellation of claims 291-296 renders the withdrawal of claims 291 and 294 moot. Applicants submit that claims 185-214 are properly grouped with claims 65-66, 93, 94 and 99, which were elected in group I, and therefore are elected subject matter.

In the restriction requirement of May 8, 2001, the Examiner listed Group I as:

> I Claims 65-66, 93, 94, 99, 100 and 187, drawn to methods for making oleic acids, linolenic acid, gamma-linolenic acid, stearidonic acid, and α-linolenic acid.

Claim 65 as restricted read:

> 65. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:
>
> growing microbial cells which contain one or more transgenes which encodes a transgene expression product under conditions whereby said one or more transgenes is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered whereby said transgene comprises a nucleotide sequence which encodes a polypeptide wherein the sequence of the polypeptide comprises a sequence selected from the group consisting of residues 50-53, 39-43, 172-172-176, 204-213 and 390-402 of SEQ ID NO: 2.

Claim 205 reads:

> 205. A method for producing a microbial cell with an altered fatty acid profile comprising:
>
> culturing a recombinant microbial cell comprising a polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2, wherein said polypeptide is expressed in sufficient amount in said cell to alter the fatty acid profile of said cell.

Applicants respectfully submit claim 205 is drawn to the elected invention. Claim 65 was part of the elected group I. Similarly, claims 189-204 and 206-214 are drawn to other variations of the invention elected with claim 65 in Group I.

As claims 185-214 are drawn to the invention elected with Group I, Applicants respectfully request reconsideration of the withdrawal of these claims. Given the inclusion of claims 65-66, 93, 94 and 99 in Group I, Applicants properly presented claims 189-214. Applicants reserve the right to petition the withdrawal of these claims, but respectfully submit that they should not be subjected to the effort and expense of doing so, as claims 189-214 are clearly within the elected invention. Consideration of claims 189-214 is respectfully requested.

The objection to claims 215-244, 255-284, 292 and 295 as dependent on nonelected subject matter

Applicants assert that these claims were previously dependent on claims properly in Group I as restricted, as set forth above. Cancellation of claims 292 and 295 renders their objection moot. Applicants have nevertheless amended the remaining claims above so that they no longer depend on subject matter that has been withdrawn from consideration. Withdrawal of the objection is requested.

The enablement rejection

Claims 225-244 and 265-284 were rejected as lacking enablement for the reasons set forth in the prior Office Action. This rejection is traversed.

The reasons set forth in the prior Office action relate to claims with 50% or 60% homology to the disclosed sequences. The claims were amended to recite sequences comprising at least 80% homology to the sequences recited in SEQ ID NOS: 1 and 2, thereby overcoming the rejections on these grounds, which are therefore inapplicable to the pending claims. Applicants further set forth the teachings regarding how to prepare sequences falling within the scope of these claims, and hereby incorporate by reference the arguments set forth in all previous responses regarding the enablement rejections.

The Training Materials For Examining Patent Applications With Respect To 35 U.S.C. Section 112, First Paragraph-Enablement Chemical/Biotechnical Applications ("Training Materials") teach that:

> There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include, but are not limited to:
>
> 1. the breadth of the claims,
> 2. the nature of the invention,
> 3. the state of the prior art,
> 4. the level of one of ordinary skill,
> 5. the level of predictability in the art,
> 6. the amount of direction provided by the inventor,
> 7. the existence of working examples, and
> 8. the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *In re Wands,* 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (reversing the PTO's determination that claims directed to methods for detection of hepatitis B surface antigens did not satisfy the enablement requirement).
>
> In *Wands*, the court noted that the there was no disagreement as to the facts, but merely a disagreement as to the interpretation of the data and the conclusion to be made from the facts. *In re Wands,* 858 F.2d at 736-40, 8 USPQ2d at 1403-07. The court held that the specification was enabling with respect to the claims at issue and found that "there was considerable direction and guidance" in the specification; there was "a high level of skill in the art at the time the application was filed;" and "all of the methods needed to

practice the invention were well known." Id. at 740, 8 USPQ2d at 1406. After considering all the factors related to the enablement issue, the court concluded that "it would not require undue experimentation to obtain antibodies needed to practice the claimed invention." Id., 8 USPQ2d at 1407.

It is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others. The examiner's analysis must consider all the evidence related to each of these factors, and any conclusion of non-enablement must be based on the evidence as a whole. Id. at 737 & 740, 8 USPQ2d at 1404 & 1407.

Training Materials at III. These factors are analyzed in turn below.

The breadth of the claims. The pending claims encompass methods using completely disclosed sequences, deletion mutants of such completely disclosed sequences, sequences with at least 80% homology to the completely disclosed sequences, and sequences which hybridize preferentially to the completely disclosed sequences. The breadth of these claims is consistent with the breadth of claims allowed by the PTO.

The nature of the invention. The claimed invention is a biotechnological invention relating to expression of polypeptides having Δ6 desaturase activity in microbial cell culture and recovery of oils and oil components therefrom. Expression of heterologous proteins in microbial cells and their use to alter metabolic processes in the host cell was known in the art for nearly two decades prior to the filing of the priority application. Isolation of oils and oil components from microbial cell cultures was also known in the art.

The state of the prior art. The state of the prior art was such that one of skill in the art was capable of conducting systematic mutagenesis analysis of every residue of a disclosed sequence and of isolating and sequencing homologous sequences using hybridization techniques and of testing such resulting mutants in a systemic fashion for over a decade prior to the instant invention without undue experimentation. Numerous reports reflect this.

The level of one of ordinary skill in the art. The level of one of skill in the art of biotechnology is high, requiring at least a Ph.D in molecular biology, biochemistry or a related discipline, and experience in generating mutants of a gene, sequencing, and expression and functional characterization of such resulting mutants.

The level of predictability in the art. The level of predictability in the art is such that a completely disclosed sequence would be expected to tolerate substitutions and deletions yielding functional mutants thereof. The level of predictability is such that a sequence which hybridized to a completely disclosed sequence could be isolated, sequenced and subject to functional characterization without undue experimentation. Recovery of oil with an altered fatty acid profile using such mutants is predictable in the art following the teachings of the disclosure.

The amount of direction provided by the inventor. As acknowledged in the Office Action at page 3, last paragraph, Applicants have provided considerable disclosure regarding methods of generating mutants and of the expression of Δ6 desaturases in microbial cells and isolation of oils with altered fatty acid profiles therefrom. SEQ ID NOS: 1 and 2 are completely disclosed. Isolation of Δ6 desaturases falling within the scope of the claims include: hybridization of the disclosed sequences to libraries from other organisms, deletion mutagenesis, insertional mutagenesis, point mutagenesis, cassette mutagenesis, site-directed mutagenesis, mutagenic PCR, conservative mutagenesis, identification by homology, and chemical mutagenesis. See, for example, page 19 line 16 through page 21 line 10 regarding the generation of mutants falling within the scope of the claims. Methods of expression and of isolating oil from microbial cultures are taught at page 22-page 30, page 32-33 and in the working examples starting at page 46 through page 66. All these methods were within the skill of the art at the time the invention was made.

The existence of working examples. Working examples of methods using the compositions of SEQ ID NOS: 1 and 2 are provided. These sequences have at least 80% homology to SEQ ID NOS: 1 and 2, respectively, and thus are working examples of such claims. A working example of the hybridization of the clone Ma524 to SEQ ID NO: 1 is provided in Example 3, and working examples of methods using SEQ ID NO: 1 are provided. No working examples are provided of methods using deletion mutants of the disclosed sequences are provided.

The Quantity Of Experimentation. Completion mutagenic analysis of known genes was within the skill of the art for more than a decade prior to the priority filing of the instant application. During that time period, advances in automated primer synthesis and automated sequencing led to huge increases in the ability to generate and analyze mutants of known sequences.

For example, a deletion analysis of a particular gene would have required at most a few months at the time of the invention. This degree of experimentation is entirely routine in the art, and in no way can be considered undue.

Similarly, complete scanning mutagenesis of a disclosed gene (including using conservative changes in amino acid sequences) as taught in the instant application at page 21 line 14 through page 22 line 10, was known in the art many years before the filing of the priority application. Cunningham and Wells described the complete alanine scanning mutagenesis of EGF years before the instant invention. One of skill in the art was aware of the techniques used in such publications. Additionally, the teachings in the instant invention further supplement the knowledge in the prior art, and render the scope of the instant claims well within the skill of the art without undue experimentation.

Applicants have disclosed many methods for generating mutants of the disclosed sequences. The Office Action has acknowledged that "mutation methods of couple of residues are taught in the specification and are well known in the prior art." Office Action, page 3, last paragraph. It is not required that Applicants teach every possible method of generating mutants. The teaching of a method of making and using one embodiment falling within the scope of the claims is sufficient to satisfy enablement. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Furthermore, if methods of making and using mutants of a couple of residues are taught in the specification and well known in the prior art, a claim to a broader genus requires only an increased scale of such teachings. Scaling up a disclosed invention using known techniques cannot be describe as undue. "[A] considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404 (citing *In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 218 (CCPA 1976)). Any experimentation used to practice the claimed invention is completely routine to one of skill in the art.

Furthermore, the settled expectations of patent applicants are paramount and are accorded great weight. *Festo Corp. v. Shoketsu Kinzoku Kogyo*, 535 U.S. 722, 152 L.Ed.2d 944 (2002). The PTO has been consistently allowing claims of equivalent scope to the claims presented here. The PTO has consistently found such scope enabled. Applicants request only that their claims be examined in accordance with the settled expectations of applicants in this field and consistently with other applications.

Accordingly, the presented claims are asserted to be fully enabled, and the application teaches one of skill in the art how to make and use the full scope of the invention. It is respectfully submitted that all of the *Wands* factors are in favor of the enablement of the pending claims. Withdrawal of the enablement rejection is respectfully requested.

The rejections under 35 U.S.C. §112, second paragraph

Claims 215-244, 255-284, 292 and 295 were rejected as indefinite on various grounds. Cancellation of claims 292 and 295 renders their rejections moot. The remaining rejections are traversed or asserted to be overcome.

(a) The Office Action stated that "the methods of claims 215, 225, 235, 255, 265 and 275 are incomplete because they are omitting essential steps." Office Action, page 4. The Office Action stated that the claims need additional steps from the claims withdrawn from consideration, and that claims 215-224 and 255-264 would be allowable if they were rewritten to include all the limitations of the base claims and any intervening claims. Office Action, page 5, third and fourth paragraphs.

Applicants thank the Examiner for the indication of allowable subject matter, and have amended claims 215, 225, 235, 255, 265 and 275 to remove their respective dependencies on claims that have been withdrawn from consideration. Claims 215-224 and 255-264 are accordingly allowable, and the indefiniteness rejection of the claims under consideration on these grounds is therefore overcome.

(b) Claim 275 was rejected as indefinite for the phrases "hybridizes to the complement of the sequence depicted in SEQ ID NO: 1" and "under hybridization conditions suitable for sequencing said complement." The arguments set forth in Applicants' previous response are incorporated by reference.

The former phrase has been amended to recite "hybridizes preferentially ..." in accordance with claim language suggested in the Training Materials For Examining Patent Applications With Respect To 35 U.S.C. Section 112, First Paragraph-Enablement Chemical/Biotechnical Applications at Example A: Analysis, which teach that the following claim is permitted:

> An isolated nucleic acid consisting of 10 to 35 nucleotides which hybridizes preferentially to RNA or DNA of bacteria A and not to non-bacteria A organisms, wherein said nucleic acid is or is complementary to a nucleotide sequence consisting of at least ten consecutive nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS 1, 2, and 3.

Emphasis added. Clearly the Patent and Trademark Office considers such language to be allowable. A nucleic acid which hybridizes preferentially to the complement of the sequence set forth in SEQ ID NO: 1 therefore would not hybridize to any other nucleic acid sequence, as alleged in the Office Action regarding the previous version of the claim. Furthermore, the claim recites the additional functional limitation regarding the specific catalytic activity of the polypeptide encoded by such nucleic acid. The metes and bounds of the phrase at issue are asserted to be clear to one of skill in the art.

The Office Action further alleges that one of ordinary skill in the art would not know what conditions are suitable for sequencing the complement of SEQ ID NO: 1. The instant application describes the sequencing of the nucleic acid set forth in SEQ ID NO: 1 (see Example 2 at page 47, esp. lines 17-29). The entire sequence of SEQ ID NO: 1, obtained in double-stranded form, is taught by Applicants. See SEQ ID NO: 1. One of skill in the art at the time of the invention could readily determine conditions suitable for sequencing both strands of SEQ ID NO:1. Kits were available for sequencing nucleic acids for over a decade prior to the instant invention. Furthermore, although not required, new claim 297 recites specific hybridization conditions found in Example 3 at page 49 lines 18-23, demonstrating the hybridization of primers used to isolate a Δ6 desaturase; the hybridization step of the PCR was carried out at 50 degrees. Claims 275 and 297 are asserted to be clear and definite to one of skill in the art. Withdrawal of the rejection of claim 275 on these grounds is respectfully requested.

(c) Cancellation of claims 292 and 295 renders the rejection regarding the phrase "substantially identical" moot.

CONCLUSION

Applicants respectfully request reconsideration of the claims in view of the above amendments and remarks. A notice of allowance is earnestly solicited. If a telephone conference would expedite allowance of this matter, the Examiner is welcome to contact the undersigned at (650) 849-4908.

If an appropriate payment does not accompany or precede this submission, the Commissioner is hereby authorized to charge any required fees, including any petition for extension of time, or to credit any overpayment, to Deposit Account No. 50-2518, billing reference no. 15611-7032 (7000934001).

Respectfully submitted,

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ATTACHMENT A

Version with markings to show changes made

IN THE CLAIMS

189. (Amended) ~~A~~ The method ~~for producing a microbial cell culture with an altered fatty acid profile comprising:~~

~~culturing a microbial cell comprising a~~ of claim 193, wherein the recombinant nucleic acid ~~comprising~~ comprises the sequence depicted in SEQ ID NO: 1 ~~to produce the microbial cell culture, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid is expressed in sufficient amount in said culture to alter the fatty acid profile~~.

193. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a microbial cell comprising a recombinant nucleic acid with at least 80% homology to the sequence depicted in SEQ ID NO: 1 to produce the microbial cell culture, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons ~~5~~ 6 and ~~6~~ 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

201. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a microbial cell comprising a recombinant nucleic acid operably linked to transcription and translation control signals functional in said cell to produce the microbial cell culture, wherein said nucleic acid is a deletion mutant of the nucleic acid depicted in SEQ ID NO: 1, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons ~~5~~ 6 and ~~6~~ 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

205. (Amended) ~~A~~ The method ~~for producing a microbial cell culture with an altered fatty acid profile comprising:~~

~~culturing a recombinant microbial cell comprising~~ of claim 208, wherein ~~a~~ the polypeptide ~~comprising~~ comprises the amino acid sequence depicted in SEQ ID NO:2 ~~to produce the microbial cell culture, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile~~.

208. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a recombinant microbial cell comprising a polypeptide with at least 80% homology to the sequence depicted in SEQ ID NO: 2 to produce the microbial cell culture, wherein said polypeptide forms a monounsaturated bond between carbons ~~5~~ 6 and ~~6~~ 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

214. (Amended) A method for producing a microbial cell culture with an altered fatty acid profile comprising:

culturing a microbial cell comprising a recombinant nucleic acid to produce the microbial cell culture, wherein said nucleic acid hybridizes preferentially to the complement of the sequence depicted in SEQ ID NO: 1 under hybridization conditions suitable for sequencing said complement, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons ~~5~~ 6 and ~~6~~ 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

215. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from ~~the~~ a microbial cell culture produced ~~according to the method of claim 189~~ by culturing a microbial cell comprising a recombinant nucleic acid comprising the sequence depicted in SEQ ID NO: 1, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid is expressed in sufficient amount in said culture to alter the fatty acid profile.

225. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from ~~the~~ a microbial cell culture produced ~~according to the method of claim 193~~by culturing a microbial cell comprising a recombinant nucleic acid with at least 80% homology to the sequence depicted in SEQ ID NO: 1, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

235. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from ~~the~~ a microbial cell culture produced ~~according to the method of claim 201~~by culturing a microbial cell comprising a recombinant nucleic acid operably linked to transcription and translation control signals functional in said cell to produce the microbial cell culture, wherein said nucleic acid is a deletion mutant of the nucleic acid depicted in SEQ ID NO: 1, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

255. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from ~~the~~ a microbial cell culture produced ~~according to the method of claim 205~~by culturing a recombinant microbial cell comprising a polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

265. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from ~~the~~ a microbial cell culture produced ~~according to the method of claim 208~~ by culturing a recombinant microbial cell comprising a polypeptide with at least 80% homology to the sequence depicted in SEQ ID NO: 2 to produce the microbial cell culture, wherein said polypeptide forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.

275. (Amended) A method for producing oil with an altered fatty acid profile comprising extracting said oil with an altered fatty acid profile from ~~the~~ a microbial cell culture produced ~~according to the method of claim 214~~by culturing a microbial cell comprising a recombinant nucleic acid to produce the microbial cell culture, wherein said nucleic acid hybridizes preferentially to the complement of the sequence depicted in SEQ ID NO: 1 under hybridization conditions suitable for sequencing said complement, said nucleic acid operably linked to transcription and translation control signals functional in said cell, wherein a polypeptide encoded by said nucleic acid forms a monounsaturated bond between carbons 6 and 7 of a fatty acid as numbered from a carboxy terminus thereof, wherein said polypeptide is expressed in sufficient amount in said culture to alter the fatty acid profile.